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PUFA and ascorbic acid combination on post-resuscitation myocardial function

Cheng Cheng[a,b,1,2], Hui Li[b,c,1,2], Lian Liang[b], Tao Jin[b], Guozhen Zhang [b], Jennifer L. Bradley[b], Mary Ann Peberdy[b,d], Joseph P. Ornato[b,e], Dayanjan S. Wijesinghe[f,g,h,1,**], Wanchun Tang[b,e,1,*]

[a] *Department of Cardiology, The Second Hospital of Anhui Medical University, Hefei, China*
[b] *Weil Institute of Emergency and Critical Care Research, Virginia Commonwealth University, Richmond, VA, USA* [c] *Department of Intensive Care Unit, The Second Hospital of Anhui Medical University, Hefei, China*
[d] *Departments of Internal Medicine and Emergency Medicine, Virginia Commonwealth University Health System, Richmond, VA, USA* [e] *Department of Emergency Medicine, Virginia Commonwealth University Health System, Richmond, VA, USA* [f] *School of Pharmacy, Virginia Commonwealth University, Richmond, VA, USA*
[g] *Institute for Structural Biology, Drug Discovery and Development, Virginia Commonwealth University, Richmond, VA, USA* [h] *Da Vinci Center, Virginia Commonwealth University, Richmond, VA, USA*

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ABSTRACT

Accumulating evidence demonstrated that administration of ω-3 polyunsaturated fatty acid (ω-3 PUFA) or ascorbic acid (AA) following cardiac arrest (CA) improves survival. Therefore, we investigate the effects of ω-3 PUFA combined with AA on myocardial function after CA and cardiopulmonary resuscitation (CPR) in a rat model.

Thirty male rats were randomized into 5 groups: (1) sham; (2) control; (3) ω-3 PUFA; (4) AA; (5) ω-3 PUFA + AA. Ventricular fibrillation (VF) was induced and untreated for 6 min followed by defibrillation after 8 min of CPR. Infusion of drug or vehicle occurred at the start of CPR. Myocardial function and sublingual microcirculation were measured at baseline and after return of spontaneous circulation (ROSC). Heart tissues and blood were collected 6 h after ROSC. Myocardial function and sublingual microcirculation improvements were seen with ω-3 PUFA or AA compared to control after ROSC ($p < 0.05$). ω-3 PUFA + AA shows a better myocardial function than ω-3 PUFA or AA ($p < 0.05$). ω-3 PUFA or AA decreases pro-inflammatory cytokines, cTnI, myocardium malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) modified proteins compared to control ($p < 0.05$). ω-3 PUFA and AA combined have lower MDA and 4-HNE modified proteins than alone ($p < 0.05$). ω-3 PUFA or AA treatment reduces the severity of post-resuscitation myocardial dysfunction, improves sublingual microcirculation, decreases lipid peroxidation and systemic inflammation in the early phase of recovery following CA and resuscitation. A combination of ω-3 PUFA and AA treatment confers an additive effect in suppressing lipid peroxidation and improving myocardial function.

1. Introduction

morbidity and mortality [1]. Overall prognosis of CA patients remains poor, even if after returning to spontaneous circulation (ROSC), and Cardiac arrest (CA) is a major public health problem with substantial myocardial dysfunction is one the main cause of early death after resuscitation [2]. Global myocardial ischemia/reperfusion (I/R) injury is considered a main cause of myocardial dysfunction after resuscitation [3].

ω-3 polyunsaturated fatty acid (ω-3 PUFA) including alpha-linolenic acid (ALA), eicosatetraenoic acid (EPA), and docosahexaenoic acid (DHA), is an essential nutrient for human health. The disorders of lipid metabolism in organs including heart occur after I/R injury following resuscitation[4,5], and supplementation with ω-3 PUFA has been shown to reduce the risk of CA [6] as well as death from sudden CA [7]. However, the effects of ω-3 PUFA and mechanisms on post-resuscitation myocardial dysfunction remain to be investigated. Ascorbic acid (AA) is an effective antioxidant. Studies have demonstrated that AA

Abbreviations: CO, cardiac output; EF, ejection fraction; MFI, microcirculatory flow index; MPI, myocardial performance index; MDA, myocardium malondialdehyde; PVD, perfused vessel density; ROSC, return of spontaneous circulation; SOD, superoxide dismutase; 4-HNE, 4-hydroxynonenal.

* Corresponding author at: Weil Institute of Emergency and Critical Care Research at VCU, Box 980266, Sanger Hall, 1101 E Marshall St, Richmond, VA, 23298- 0279, USA.
** Corresponding author at: School of Pharmacy, Virginia Commonwealth University, Richmond, VA, USA. *E-mail addresses:* wijesingheds@vcu.edu (D.S. Wijesinghe), wanchun.tang@vcuhealth.org (W. Tang).
[1] Equal contribution.
[2] Co-first authors.

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administration at the start of cardiopulmonary resuscitation (CPR) decreased myocardial damage and improved survival rate and neurological outcome in a rat model of cardiac arrest and CPR [8,9]. Current evidence further indicates that the combination of two or more antioxidants may exert synergistic myocardial protective effects [10,11].

The present study investigates the myocardial protective effects and underlying mechanisms of ω-3 PUFA following CA in a rat model of CPR. We hypothesize that ω-3 PUFA suppresses oxidative damage, inflammation, reduces the severity of post resuscitation myocardial and microcirculatory dysfunction. We further investigate whether the combination ω-3 PUFA and AA may provide additional protection of myocardial function.

2. Methods

2.1. Animal preparation

Male Sprague Dawley rats, weighing between 450− 550 g were anesthetized by intraperitoneal injection of pentobarbital (45 mg/kg), and additional doses (10 mg/kg) were administered each hour or as required to maintain anesthesia. The trachea was orally intubated with 14-G cannula mounted on a blunt needle (Abbocath-T; Abbott Hospital Products Division) with a 145-degree angled tip. End-tidal CO_2 ($ETCO_2$) was continuously monitored with a side-stream infrared CO_2 analyzer (Capstar-100 Carbon Dioxide Analyzer; CWE, Ardmore, PA). A conventional lead II electrocardiogram (ECG) was continuously monitored.

A polyethylene catheter (PE-50; Becton Dickinson, Sparks, MD) was placed into the descending aorta from the left femoral artery to measure aortic pressure, and another PE-50 catheter was advanced into left external jugular vein to measure right atrial pressure. Arterial and right atrial pressure were monitored by high-sensitivity transducers (model 4284-01; Abbott Critical Care System). Another PE-50 catheter was inserted into the right femoral vein for drug administration. A thermocouple microprobe (IT-18; Physitemp Instruments, Clifon, NJ) was placed into the left femoral vein to detect temperature. A 3 F PE catheter (model C-PMS-301 J; Cook Critical Care, Bloomington, IN) was advanced through the right external jugular vein into the right atrium for inducing ventricular fibrillation (VF). A pre-curved guidewire supplied with the catheter was then advanced through the catheter into the right ventricle. All catheters were flushed intermittently with saline containing crystalline bovine (2.5 IU/mL). The core temperature was maintained at 36.5–37.5 °C by a heating blanket and adjusting the distance between animals and the heating lamp.

2.2. Experimental procedures and treatment

Rats were randomized into 5 groups (n = 6): (1) sham (2) control (3) ω-3 PUFA (4) Ascorbic acid (AA) (5) ω-3 PUFA + AA (ω-3 PUFA + AA).

Fifteen minutes prior to induction of VF, baseline data and echocardiography were measured. Mechanical ventilation was set at a tidal volume of 0.60 mL/100 g of body weight, a frequency of 100 breaths/ min, and F_{IO2} of 0.21. VF was then induced through a guidewire advanced from the right jugular vein into the right ventricle. A progressive increase in 60-Hz current to a maximum of 3.5 mA was then delivered to the right ventricular endocardium. The current flow was continued for 3 min to prevent spontaneous defibrillation. Mechanical ventilation was suspended once VF onset. After 6 min of untreated VF, mechanical ventilation (tidal volume 0.60 mL/100 g body weight, frequency 100 breaths/min, and F_{IO2} 1.0) was restarted and precordial chest compression was initiated using a pneumatically driven mechanical chest compressor. The rate of compression was maintained at 200/ min and synchronized to provide a compression/ventilation ratio of 2: 1 with equal compression-relaxation for 8 min. Defibrillation was then attempted with up to three 4-J counter. If resuscitation was not successful after the electrical shock, precordial compression was resumed for 30 s, followed by another sequence of defibrillation. Return of spontaneous circulation (ROSC) was defined as the return of supraventricular rhythm with a mean aortic pressure over 50 mmHg for 5 min. Mechanical ventilation and hemodynamic measurements were continued for 6 h after ROSC. The F_{IO2} of 1.0 was continued for 1 h, and then adjusted to 0.5 for the second hour, and 0.21 thereafter[12].

For the sham group, animals were operated exactly as the other groups except without VF and CPR. Saline placebo, ω-3 PUFA (Omegaven; Fresenius Kabi, Bad Homburg, Germany, 5 mL/kg)[13] or AA (Sigma Aldrich, A5960,100 mg/kg) [8] was infused in an amount of 1.25 mL/(kg h at the start of CPR over a period of 4 h with an infusion pump. In ω-3 PUFA + AA group, AA (100 kg/kg) was fully dissolved in ω-3 PUFA (5 mL/kg) and continues intravenous injection at the start of CPR for 4 h. 1.0 mL of blood was drawn at baseline and 6 h after ROSC. Animals were sacrificed and heart tissues were obtained at 6 h after ROSC. The serum samples were collected for ELISA assays, and the hearts were appropriately disposed of for further assays.

2.3. Hemodynamics measurements

ECG, aortic and right atrial pressures, $ETCO_2$ and temperature values were continuously recorded on a personal computer-based data-acquisition system supported by WINDAQ software (DATAQ). Coronary perfusion pressure (CPP) was calculated as the difference in the time- coincident diastolic aortic and right atrial pressures that were displayed in real-time. *2.4. Echocardiography measurements*

Ejection fraction (EF), cardiac output (CO), and myocardial performance index (MPI) were measured by echocardiography (HD 11XE; Phillis Medical Systems) with a 12.5-Hz transducer at baseline, 1, 3, and 6 h after ROSC. CO and EF were used to evaluate left ventricular systolic function; MPI was the indicator of both myocardial systolic and diastolic function[14]. All measurements were reviewed and confirmed separated by two investigators blinded to the groups.

2.5. Sublingual microcirculation measurements

Sublingual microcirculation was measured at baseline, 2, 4 and 6 h after ROSC using side-stream dark-field imaging device (MircroScan; MicroVision Medical, Amsterdam, the Netherlands) that had a 5× imaging objective, resulting in an on-screen magnification of 276 × . Three discrete fields for each were captured with the intention to minimize motion artifacts. Microvascular images were recorded on a DVD with a DVD recorder (DMR-EZ47 V; Panasonic AVC Networks, Dalian, China) [12]. Microcirculatory flow index (MFI) was to estimate blood flow in the small vessels, which were less than 20 μm in diameter. Briefly, the image was divided into four quadrants, and the predominant flow was classified into 4 grades: absent = 0, inter intermittent = 1, sluggish = 2, normal = 3. The MFI score represented the average values of the four quadrants [15]. Perfused vessel density (PVD) was calculated as the number of vessels crossing in the catheters divided by the

total length of the catheters [16]. All recordings were analyzed by three independent observers blinded to the groups.

2.6. ELISA

TNNI3/Cardiac Troponin I (cTnI), interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) levels in serum were measured with ELISA kit in accordance with the manufacturer's instructions (category NO. LS- F23616, LSBio; category NO.R6000B, R&D SYSTEM; category NO. RTA00, R&D SYSTEM), respectively. Enzymatic activity was determined spectrophotometrically in absorbance at 450 nm using a 96-well plate reader. The detection limit for cTnI, IL-6 and TNF-α was 31.25, 62.5 and 15.6 pg/mL, respectively.

2.7. Superoxide dismutase (SOD) and malondialdehyde (MDA)) assays in heart tissues

Heart tissues were harvested, frozen immediately and stored at − 80 °C until assessment. SOD activity of heart was determined using superoxide dismutase Assay Kit (No.706002, Cayman), according to the manufacture's protocol. Heart samples were homogenized in HEPES buffer at a ratio of 1:5 (weight: volume), and then the homogenate was centrifuged at 1500 *g* for 5 min at 4 °C. Followed by the protocol, the absorbance value of each sample was determined at 450 nm.

The levels of MDA in heart samples were measured using thiobarbituric acid reacting substances (TBARS) Assay Kit (No. 10009055, Cayman), according to the manufacture's protocol. Briefly, tissues from the heart were homogenized in RIPA buffer at a ratio of 1:10 (weight: volume). The homogenate was centrifuged at 1600 *g* for 10 min at 4 °C. The lipid peroxide level in the supernatant was measured followed by the protocol, and MDA contents were assessed by TBRAS at a wavelength of 530 nm. *2.8. Western blotting*

Heart tissues were homogenized in RIPA buffer consisting of protease inhibitor cocktail (Roche) and phosphatase inhibitor PhosSTOP. The homogenate was centrifuged at 12000 rpm for 15 min at 4 °C, and the supernatant was extracted for further protein quantitation. Thirty micrograms of protein for each sample was separated on 4–20 % SDS/PAGE gel electrophoresis and transferred to PVDF membrane. The selected membrane was blocked with 1×Tris Buffer Saline (TBS) with 1% Casein for 1 h at room temperature with 120 bpm shaking. Subsequently, the membrane was incubated with primary antibodies at a dilution 1:1000 to 1:2000 overnight at 4◦C. 4-HNE (Ab#46545, Abcam) was used as the primary antibody. GAPDH (Ab#8245, Abcam) was performed as an internal control. After washing with 1 × TBST, the membrane was incubated with secondary anti-rabbit/mouse IgG for 1 h at room temperature. The protein quantitative analysis was done using ImageJ software.

2.9. Statistical analysis

Statistical analysis was performed using version 24.0 (SPSS Inc., Chicago, Illinois, U.S.A.). Data were presented as mean ± SD. The two- factor repeated-measures ANOVA followed by Tukey post hoc test was used to analyze the differences of myocardial function (EF, CO and MPI), sublingual microcirculation (PVD and MFI), and serum index amongst groups. One-way ANOVA followed by Bonferroni post hoc test was used to analyze the differences of SOD, TBARS and expression levels of 4-HNE protein amongst groups. *p*-value <0.05 was considered significant.

3. Results

3.1. The effects of ω-3 PUFA and AA on post-resuscitation myocardial function

There was no difference in myocardial function (EF, CO and MPI) among the 5 groups at baseline. However, significant impairment in myocardial function was seen following resuscitation in all animals that underwent cardiac arrest and CPR (Fig. 1). Impairment of EF, CO and MPI were improved in ω-3 PUFA, AA, and ω-3 PUFA + AA compared to control (Fig. 1**A**-**B**). Moreover, combined treatment of ω-3 PUFA and AA led to a further amelioration in myocardial function compared to ω-3 PUFA or AA alone. No differences were seen between ω-3 PUFA and AA (Fig. 1).

3.2. The effects of ω-3 PUFA and AA on post-resuscitation on sublingual microcirculation

Sublingual microcirculation including MFI and PVD was impaired significantly in all animals after resuscitation. However, 2 h following resuscitation, significantly lesser impairment of sublingual microcirculation was observed in animals treated with the combination of ω-3

PUFA + AA when compared with control animals. All animals treated with either ω-3 PUFA or AA exhibited a better sublingual microcirculation than the control animals after 4 h of ROSC. However, there were no significant differences in microcirculation among the ω-3 PUFA + AA group and the single drug-treated groups at all time points (Fig. 2).

3.3. ω-3 PUFA and AA attenuated inflammation and myocardial injury

Plasma IL-6 and TNF-α levels were significantly increased 6 h after ROSC in all animals following resuscitation. However, ω-3 PUFA or AA significantly reduces systemic inflammatory response compared to control. A combination of ω-3 PUFA and AA treatment further reduces post-resuscitation systemic inflammatory response, but there was no significance (Fig. 3**A** and **B**). Importantly, plasma cTnI was also

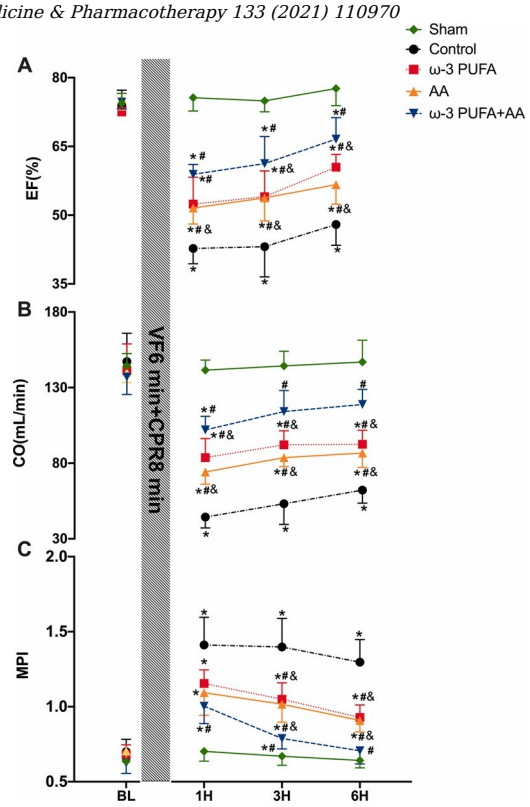

Fig. 1. ω-3 PUFA and AA effects on post-resuscitation myocardial function. (**A**) Ejection fraction, (**B**) Cardiac output, (**C**) Myocardial performance index. CO, cardiac output; EF, ejection fraction; MPI, myocardial performance index; BL, baseline; VF, ventricular fibrillation; CPR, cardiopulmonary resuscitation. Data were presented as means ± SD (n = 6 in each group). *$p < 0.05$ versus sham. #$p < 0.05$ versus control.
&$p < 0.05$ versus ω-3 PUFA + AA group.

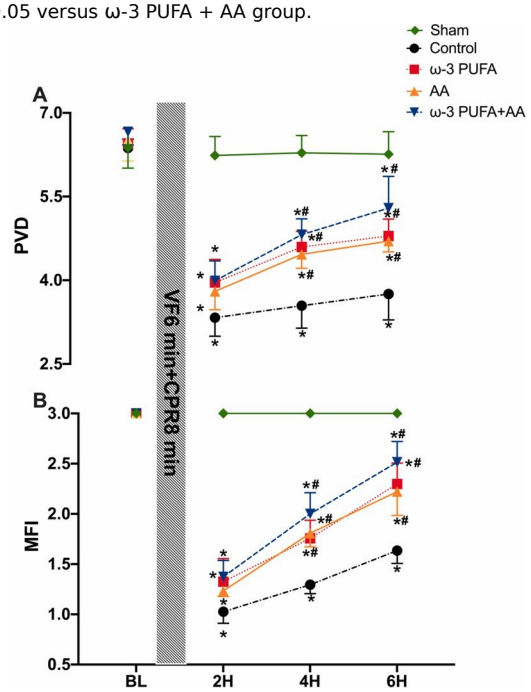

Fig. 2. ω-3 PUFA and AA effects on post-resuscitation sublingual microcirculation.
(**A**) Perfused vessel density, (**B**) Microcirculatory flow index. PVD, perfused vessel density; MFI, microcirculatory flow index; BL, baseline; VF, ventricular fibrillation; CPR, cardiopulmonary resuscitation. Data were presented as mean ± SD (n = 6 in each group). *$p < 0.05$ versus sham. #$p < 0.05$ versus control.

markedly increased in control at 6 h after ROSC. Plasma cTnI levels are decreased with ω-3 PUFA and AA treatment. No significant decrease of cTnI was noted between ω-3 PUFA + AA and single-drug groups
(Fig. 3**C**).

3.4. ω-3 PUFA and AA suppress oxidative stress, and combination of ω-3 PUFA and AA mitigated lipid peroxidation of the myocardium

SOD, MDA, and 4-HNE modified proteins in heart tissues were analyzed for evaluating oxidative stress. Compared with sham- operation, resuscitation caused a dramatic reduction of SOD activities and a substantial increase of TBARS levels in hearts. Compared to sham, resuscitation causes a dramatic reduction of SOD activity and a substantial increase of TBARS levels within the heart. ω-3 PUFA treatment increases SOD activity however AA has no effect on SOD (Fig. 4**A**). Furthermore, both ω-3 PUFA and AA reduced the levels of TBARS contents in rats undergoing resuscitation (Fig. 4**B**). The expression of 4-HNE modified proteins were markedly increased by CA (Fig. 4**C-D**). Nevertheless, treatment with ω-3 PUFA, AA, and ω-3 PUFA + AA significantly reduced TBARS levels and 4-HNE modified proteins content in resuscitated rats. The rats treated with ω-3 PUFA combined with AA showed the lowest levels of TBARS and 4-HNE modified proteins among the three treatment groups (Fig. 4**B**–**D**).

4. Discussion

In the present study, we demonstrated that administration of ω-3 PUFA or AA at the start of CPR significantly alleviated system inflammation, oxidative stress, and myocardial injury, which may contribute to enhanced cardioprotection and microcirculation. We further demonstrated that the combination of ω-3 PUFA and AA provided better protection of myocardial function than a single drug. These benefits are likely due to the alleviation of lipid peroxidation by the synergy effect from combining the two drugs.

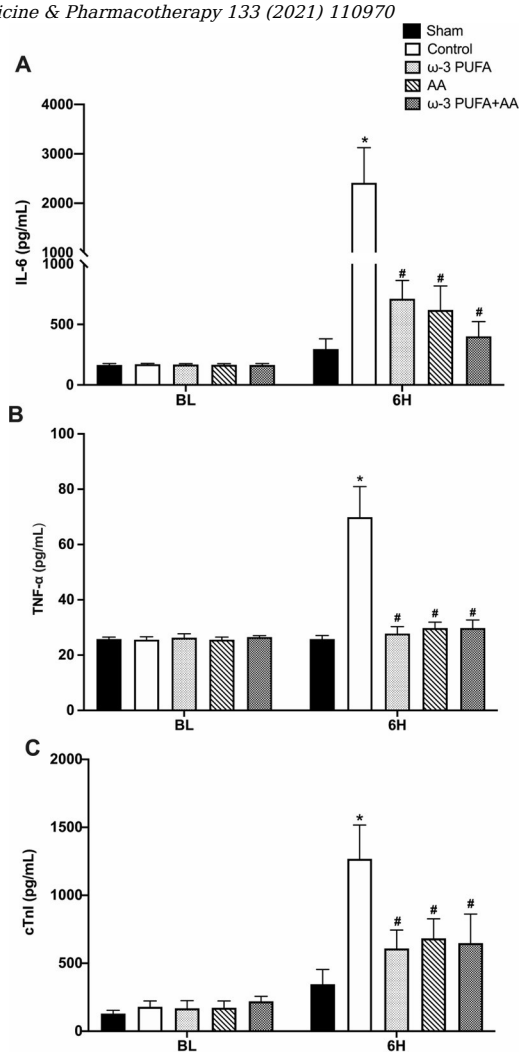

Fig. 3. ω-3 PUFA and AA effects on post-resuscitation pro-inflammatory cytokines and cTnI. (**A**) Plasma levels of IL-6, (**B**) Plasma levels of TNF-α, (**C**) Plasma levels of cTnI. BL, baseline; IL-6, interleukin 6; TNF-α, tumor necrosis factor- α; cTnI, TNNI3/Cardiac Troponin I. Data are presented as mean ± SD. $*p < 0.05$ versus sham. $\#p < 0.05$ versus control.

As our results revealed, ω-3 PUFA treatment reduced the severity of post-resuscitation myocardial dysfunction. The cardioprotective effects of ω-3 PUFA result from multiple and complex mechanisms that including antiarrhythmic, modulation of cardiac ion channels, lower resting heart rate and blood pressure, and improvement of myocardial filling and vascular function[17]. Several studies have indicated ω-3 PUFA improves the isolated perfused cardiac function through anti-inflammatory, anti-oxidation, attenuating mitochondrial damage, and restoring mitochondrial respiratory activities, whether pre-treated or post-treated by ω-3 PUFA[18,19]. It has been proved that sublingual microcirculation was correlated with severity of post-resuscitation myocardial dysfunction[20]. In the present study, ω-3 PUFA treatment significantly increased PVD and MFI values compared with control group at 4 h and 6 h after ROSC, which indicates that ω-3 PUFA may ameliorate microcirculation defects through improved myocardial function. There was a temporal discordance between the improvement of microcirculation and myocardial function after CPR. The reason may be that a reduction in microcirculation of peripheral tissues due to vasoconstriction occurred to preserve perfusion to vital organs under the condition of insufficient systemic blood flow[21].

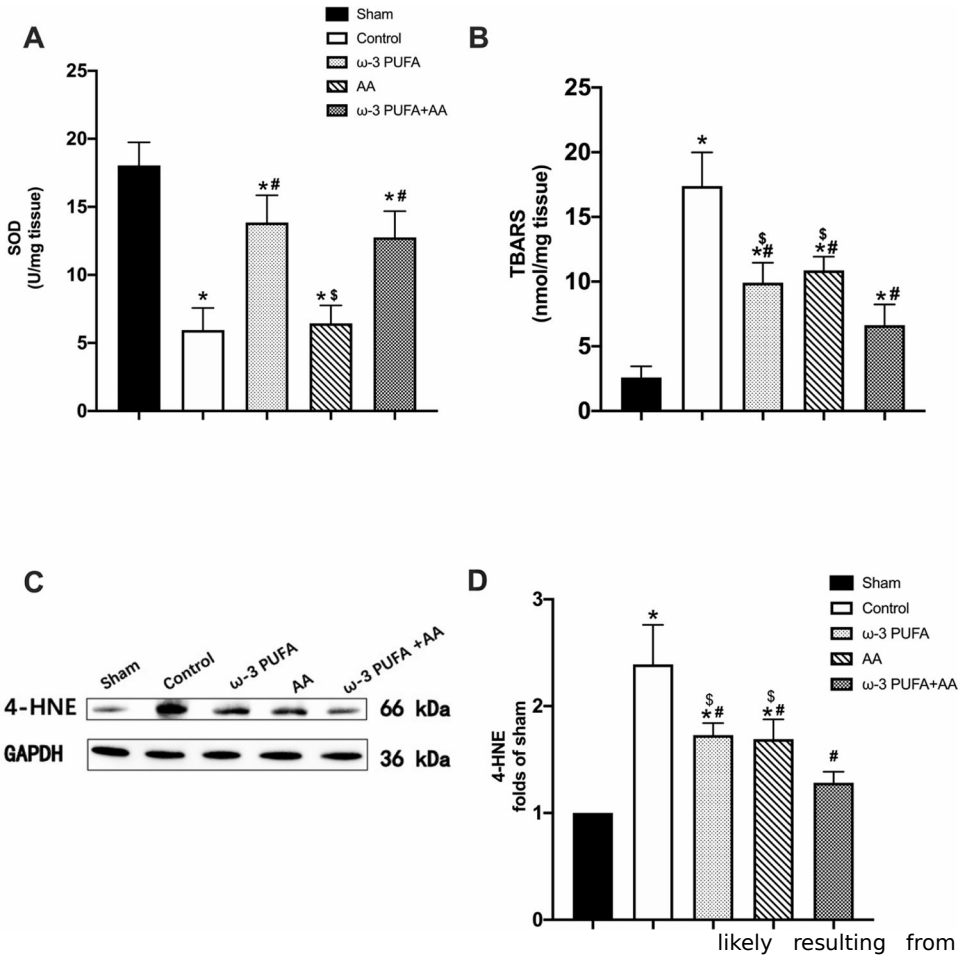

Fig. 4. ω-3 PUFA and AA effects on oxidative damage in heart tissues. (**A**) Heart SOD activities (**B**) Heart TBARS levels (**C**) Representative protein bands of 4-HNE modified proteins and GAPDH. (**D**) Quantification of the expression levels of 4-HNE modified proteins in heart tissue. BL, baseline; SOD, superoxide dismutase; TBARS, thiobarbituric acid reacting substances; 4-HNE, 4-hydroxynonena. Data are presented as mean ± SD. *p < 0.05 versus sham. #p < 0.05 versus control. $$p < 0.05$ versus ω-3 PUFA + AA.

The improvement of myocardial function and microcirculation following the treatment of ω-3 PUFA are most likely resulting from reduced ischemia-induced oxidative stress and systemic inflammatory response. Oxidative stress during global I/R is one of the primary mechanisms of post-resuscitation myocardial dysfunction. ω-3 PUFA has been reported to attenuate oxidative stress in cardiac tissue, and the antioxidant effects mainly contribute to its incorporation into the cell membrane and the modulation of the antioxidant signaling pathway [18]. Consistent to these studies, our results demonstrated that ω-3 PUFA treatment significantly reduced the levels of MDA (a lipid peroxidation marker), and increased the activity of SOD (a natural antioxidant marker) in the myocardium compared with those in control animals, suggesting ω-3 PUFA attenuated the oxidative damage and enhanced the effects of anti-oxidation.

Cardiac biomarkers indicate the severity of myocardial injury and prognosis in the early stage of postresuscitation. Our findings showed that the levels of cTnI were significantly elevated in all animals following cardiac arrest and CPR, while ω-3 PUFA treatment significantly decreased the myocardial injury. Recent clinical evidence suggests that activation of inflammatory cascade has critical roles in I/R injury after resuscitation [22]. Therefore, reducing the systemic inflammation contributes to ameliorate cardiac damage after CA [23]. Mechanisms underlying the anti-inflammatory actions of ω-3 PUFA include altered cell membrane phospholipid fatty acid composition, disruption of lipid rafts, and inhibition of NF-κB pathway [24]. Our study demonstrated ω-3 PUFA treatment dramatically reduced the production of pro-inflammatory cytokines, which may protect myocardial I/R

injury following CPR. In has been reported ω-3 PUFA reduces the number of rolling leukocytes and prevent the rise of leukocyte adhesion during I/R, thereby protecting microvascular [25]. This was supported by our results that ω-3 PUFA improved sublingual microcirculation following CPR.

As a strong antioxidant, AA is essential to anti-oxidation of human body, and reducibility is the representative feature. The reduction of AA eliminates the toxicity of ROS. Its antioxidant effect is manifested in its rapid reaction with O2-, HOO- and OH- to generate semi- dehydroascorbic acid, scavenging singlet oxygen, and reducing sulfur- free radicals. Therefore, both SOD and AA have the effect of scavenging ROS. In two studies of CA rat model, AA treatment significantly reduced MDA, decreased myocardial damage, and improved survival rate [8,9]. Our results are supported by these studies. In the present study, we found AA treatment at the start of CPR decreased system inflammation, lipid peroxidation (MDA and 4-HNE modified proteins) and tissue injury in the myocardium (cTnI) (Fig. 2), and thus significantly improved myocardial function since early post-resuscitation and continued to the end of the procedure (Fig. 1). Furthermore, AA treatment improved sublingual microcirculation at 4 h and 6 h after ROSC compared to the untreated group in our study. Oxidative stress after post-resuscitation results in endothelial cells swelling, leukocytes adhering to the exothelium and microthrombi formatting, and thus impairing microcirculatory flow. By reducing leukocyte plugging in microvessels and preventing endothelial cell swelling, AA decreases microcirculatory flow impairment [26,27]. AA was applied as a single bolus or as a continuous infusion, sometimes preceded by a bolus. Because of the half-life of parenteral AA is short, bolus AA will reach effectively the blood concentration for only a short period. In our study, animals treated with AA at the start of CPR and continuous infusion for 4 h, so the benefits were exhibited since the early post-resuscitation.

Previous studies reported beneficial effects of the combination of AA and ω-3 PUFA on antioxidation after I/R [28–30]. We demonstrated a significant improvement of post-resuscitation myocardial function in animals treated with the combination of AA and ω-3 PUFA compared with monotherapy with AA or ω-3 PUFA. Our study further demonstrated that the expressions of MDA and 4-HNE modified proteins in rats treated with the combination were the lowest among the post-resuscitation groups. These findings suggested that the combination of AA and ω-3 PUFA enhanced myocardial function and alleviated myocardial injury might through reducing lipid peroxidation. AA has been found to suppress peroxidation of PUFA [31,32]. In the case of ω-3 PUFA combined with lipid peroxidation inhibitor, the peroxidation of ω-3 PUFA reduced and the effects of ω-3 PUFA enhanced [29]. Thus, it might explain the significant improvement of myocardial function in ω-3 PUFA + AA group. However, we did not find a significant improvement in sublingual microcirculation in ω-3 PUFA + AA group compared with ω-3 PUFA or AA group, indicating that there may be other mechanisms involved in post resuscitation microcirculatory dysfunction.

There are some limitations to the study. First, only 6 h after ROSC as the duration of observation, the effects of the interventions on neurological function and survival were not investigated. Second, animals without any underlying diseases were studied, which is not consistent with clinical reality. At last, the plasma levels of ω-3 PUFA and AA were not measured, thus the most effective doses of ω-3 PUFA and AA benefited to myocardial function were not able to estimate.

5. Conclusion

Our present study suggests that ω-3 PUFA or ascorbic acid administration at the start of CPR decreases lipid peroxidation and systemic inflammation, improves post-resuscitation myocardial function and sublingual microcirculation in the early phase of recovery following CA and resuscitation. A combination of ω-3 PUFA and ascorbic acid treatment provides an additive effect in improving myocardial function and suppressing lipid peroxidation.

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Disclosures

None.

Declaration of Competing Interest

The authors report no declarations of interest.

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